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# Chewing areca nut, betel quid, oral snuff, cigarette smoking and the risk of oesophageal squamous-cell carcinoma in South Asians: A multicentre case-control study

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## ABSTRACT

Oesophageal cancer remains an important public health problem worldwide. This multicentre matched case-control study examined the chewing areca nut alone, betel quid with tobacco, oral snuff (snuff dipping) and cigarette smoking as the risk factors for oesophageal squamous-cell carcinoma. We enrolled 91 cases of oesophageal squamous-cell carcinoma and 364 matched controls from three tertiary-care hospitals in Karachi, Pakistan. A structured questionnaire was used to collect the data through face-to-face interview of the participants. Multivariable conditional logistic regression model showed that after adjusting for the effect of ethnicity, ever chewed areca nut alone (adjusted matched odds ratio (mOR<sub>adj</sub>) = 3.7; 95% confidence interval (CI): 1.6–8.5), ever chewed betel quid with tobacco (mOR<sub>adj</sub> = 12.8; 95% CI: 6.3–26.2), ever practiced snuff dipping (mOR<sub>adj</sub> = 4.3; 95% CI: 1.6–11.7) and ever smoked cigarettes (mOR<sub>adj</sub> = 2.9; 95% CI: 1.4–5.9) were significantly and independently associated with oesophageal squamous-cell carcinoma status. The adjusted summary population attributable risk (PAR) percent for all four substances together was 67.0. Furthermore, despite incomplete synergy, there was manifold increase in the risk of oesophageal squamous-cell carcinoma, if the respondents ever smoked cigarettes and ever chewed betel quid with tobacco (mOR<sub>adj</sub> = 21.4; 95% CI: 6.3–72.4) or if they ever smoked cigarettes and ever practiced snuff dipping (mOR<sub>adj</sub> = 14.4; 95% CI: 2.3–91.1). The adjusted PAR (%) was higher for the dual practice of smoking cigarettes and chewing betel quid with tobacco (64.3) than the dual practice of smoking cigarettes and snuff dipping (32.2). Public awareness to curtail the addiction to these substances may result in a substantial reduction in the incidence of oesophageal squamous-cell carcinoma and related mortality in this and similar settings.

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## 1. Introduction

Oesophageal cancer remains an important public health problem worldwide. It is currently the 8th most common human cancer and 6th most common cause of cancer-related

deaths.<sup>1</sup> In 2005, the worldwide estimated burden of oesophageal cancer included estimated 500,000 new cases and 416,500 related deaths. Of the new cases of oesophageal cancer and related deaths, 84% occurred in less developed countries particularly in Asia.<sup>2</sup> Oesophageal cancer is two to

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four times more common among men than women.<sup>1</sup> By contrast, in Karachi, Pakistan, between January 1, 1995 and December 31, 2002, age-standardised rates (per 100,000 populations) of oesophageal cancer were higher in women (8.6) than in men (6.4).<sup>3</sup>

The most common histologic types of oesophageal cancer are squamous cell carcinoma and adenocarcinoma, which together constitute more than 90% of oesophageal malignancies. These two major histologic types of oesophageal carcinoma differ substantially in their underlying incidence patterns and key aetiological factors thus complicating the prevention.<sup>4</sup> Notwithstanding a recent increase in the incidence of oesophageal adenocarcinoma in western populations,<sup>4–6</sup> globally oesophageal squamous cell carcinoma is the most common (90%) subtype, specifically in Eastern countries,<sup>7</sup> including Pakistan (86–87%).<sup>8,9</sup> The risk factors for oesophageal squamous cell carcinoma including low-income, cigarette smoking, high-alcohol intake, low intake of raw fruits and vegetables, ingestion of coarse and raw foods are relatively better understood in western populations and together may account for up to 98% of the oesophageal carcinoma cases.<sup>7</sup> However, there are limited published data on the role of these risk factors and the chewing of smokeless tobacco and areca nut in causation of oesophageal squamous-cell carcinoma in South Asia, specifically in Pakistan.

Consumption of various forms of smokeless tobacco is an integral cultural tradition in South Asia with its prevalence up to 21% in general population.<sup>10–12</sup> Smokeless tobacco is chewed as betel quid, gutka, oral snuff (snuff dipping) and/or administered through nose (nasal snuff).<sup>13</sup> Betel quid is a mixture of areca nut, slaked lime (aqueous calcium hydroxide paste), with or without tobacco, condiments and with and without sweeteners wrapped in a betel leaf. It is chewed and held in the mouth like a quid. Gutka is powered mixture of areca nut, tobacco, slaked lime, sandalwood and fragrance. Oral snuff is a moist mixture of tobacco and lime. Chewing of areca nut alone is also a widely practiced addiction in South and South-East Asian populations.<sup>13,14</sup>

In general, these orally consumed products are highly addictive, and typically contain several carcinogens that cause head, neck and throat cancers with high proportional premature mortality.<sup>15</sup> Smokeless tobacco-specific nitrosamines are believed to be the most strong carcinogens and play a significant role in the aetiology of oral cancer.<sup>16</sup> However, the role of smokeless tobacco in oesophageal squamous-cell carcinoma is not well documented. Furthermore, poor prognosis of oesophageal cancer patients (with only 5–10%, 5-year survival rates) calls for primary prevention as the desired goal. Therefore, this multicentre matched case-control study examined the use of chewable substances (areca nut alone, betel quid with tobacco, and oral snuff) and cigarette smoking as the risk factors for oesophageal squamous-cell carcinoma in urban Pakistan.

## 2. Participants and methods

### 2.1. Study design and settings

Between January 1998 and December 2002, we conducted a matched case-control study at three tertiary care teaching

hospitals in Karachi. Karachi is the largest and a cosmopolitan city with a multi-ethnic 18 million population. It is a major economic hub of the country and has an influx of workers from all over the country. Therefore, people of different ethnicities identified based on their mother tongue live and work together in various neighbourhoods in the city. These different ethnic groups tend to vary in their traditions, cultural values, dietary patterns and perhaps in the use of various substances considered in this study. The study hospitals included Aga Khan University Hospital (AKUH), Civil Hospital Karachi (CHK) and Liaquat National Postgraduate Medical Center (LNPMC) and provide care to over 3500 inpatients belonging to various socioeconomic strata of the city. Patients attend these hospitals from all over the Sindh – the second largest province of Pakistan with a population of 30 million. This study was restricted to permanently domiciled residents of Karachi.

### 2.2. Selection of cases and controls

Cases were patients of any age and sex with biopsy proven oesophageal squamous-cell carcinoma diagnosed at any of the three study hospitals. Patients with adenocarcinoma or any other tumour of the oesophagus were excluded. Controls were inpatients admitted for a wide spectrum of acute conditions but free from oesophageal squamous-cell carcinoma. Four controls matched for age ( $\pm 2$  years) and sex with each case were selected from the same hospital as the case. Controls were excluded, if they had any other malignant lesion or past history of any malignancy. We also excluded controls from respiratory, cardiology and gynaecological wards or people with complaints of dysphagia. A biopsy report confirming the absence of oesophageal squamous-cell carcinoma was not required. Performing a biopsy on all controls was not logistically feasible or acceptable.

### 2.3. Exposure assessment

A structured questionnaire was used to collect information from each selected case and control through face-to-face interview in Urdu. Data on socio-demographic variables and various substance uses were assessed following the definitions used previously.<sup>17</sup> Questions were asked about the lifetime chewing areca nut alone, chewing betel quid with or without tobacco, gutka, snuff dipping, cigarette smoking or other methods of tobacco smoking (i.e. cigars, hookah, pipe, bidi) and alcohol drinking. Chewable substance users were defined as the individuals who chewed areca nut alone, chewed betel quid with or without tobacco, gutka, or practiced snuff dipping at least once a day for minimum 1 year. Smokers were persons who had ever smoked cigarettes, bidi, hookah, cigar, or a pipe daily for at least 1 year. Those who smoked at that time were referred to as current smokers, while ex-smokers were those who had stopped smoking 2 or more years before the date of diagnosis or interview. The participants who had drunk an alcohol beverage (beer and wine) more than four times a week for at least 1 year were defined as ever alcohol drinkers. The quantity and the duration of the use of these substances were also assessed. Informed consent was taken from each participant before the interview,

and assured about the confidentiality of collected information. The institutional Ethics Review Committee approved the study protocol.

#### 2.4. Sample size

For this matched case–control study, we estimated 95 cases and 380 controls (case:control ratio = 1:4), assuming the users of betel quid with tobacco were at least three times as likely to develop oesophageal squamous-cell carcinoma as non-users and that the prevalence of chewing betel quid with tobacco in this population was 10%. We further assumed 90% study power, 5% type 1 error and correlation coefficient of 0.2 for chewing betel quid with tobacco between matched cases and controls.

#### 2.5. Statistical analysis

Means and standard deviations (SD) of quantitative variables and frequencies (%) of categorical variables were computed for cases and controls. Before further analysis, based on the composition of various preparations and relative frequencies of their users, the individuals who used betel quid without tobacco or used areca nut alone were combined as ‘ever users of areca nut alone’. The individuals who used betel quid with tobacco or used gutka were combined as ‘ever users of betel quid with tobacco’. Furthermore, bidi smokers, current or ex-cigarettes smokers were grouped together as ‘ever cigarette smokers’. However, snuff dipping was treated as a separate exposure being a distinct practice in some segments of the South Asian populations. Univariable conditional logistic regression analysis was used to evaluate socio-demographic variables and hypothesised risk factors for their unadjusted associations with oesophageal squamous-cell carcinoma status.<sup>18</sup> Variables related ( $p \leq 0.15$ ) in univariable analyses were further examined in multivariable conditional logistic regression models for their independent associations with oesophageal squamous-cell carcinoma status. A variable was included in the final multivariable model, if it made at least 10% change in the 95% confidence interval (CI) for an effect estimate of one or more variables already in the model. After fitting the main effects model, two-way multiplicative interactions were examined by computing the joint effects of ‘cigarette smoking with chewing betel quid with tobacco’, ‘cigarette smoking with chewing areca nut alone’ and ‘cigarette smoking with snuff dipping’. When the joint effects greater than the expected under multiplicative models were not observed, additive interactions were evaluated. We computed relative excess risk (RERI), attributable proportion (AP) and synergy index (SI) by using multivariable conditional logistic regression model coefficients and their variance–covariance matrix.<sup>19</sup> Adjusted matched odds ratios (mOR<sub>adj</sub>) and their 95% CIs as the approximation of the relative risks were used for interpretation of the final model. Furthermore, by using the parameters’ estimates for the risk factors in the final model, the population attributable risk (PAR) percent of oesophageal squamous-cell carcinoma for each risk factor and summary PAR (%) for all the risk factors together were computed.<sup>20</sup> Also, the PARs (%) of the oesophageal squamous-cell carcinoma for the dual practice of smoking ciga-

rettes with chewing betel quid with tobacco and the dual practice of smoking cigarettes with snuff dipping were computed.<sup>20</sup>

### 3. Results

Of the 93 eligible, we enrolled 91 cases of oesophageal squamous cell carcinoma and 364 controls from the three study hospitals. Two male cases did not participate in the study either because of the advanced cancer stage (1) or simply refused without citing any reason (1). None of the identified controls declined to participate in study. The mean ( $\pm$ SD; range) age (completed years) of cases and controls was 54.4 ( $\pm$ 13.4; range: 22–90) and 54.2 ( $\pm$ 13.9; range: 20–90), respectively. The proportions of cases and controls were about the same at each level of educational attainment. A substantial proportion of cases (42.9%) and controls (42.0%) were housewives. More cases (23.1%) than controls (16.2%) were medium earners. However, more controls (34.3%) than cases (25.3%) were low earners. Among cases compared to controls, higher

**Table 1a – Comparison of the socio-demographic characteristics and exposures recorded on the participants in a multicentre matched case–control study of squamous cell carcinoma of oesophagus, Karachi, Pakistan.**

Socio-demographic characteristics	Cases (n = 91)		Controls (n = 364)	
	n	%	n	%
<i>Hospital<sup>a</sup></i>				
AKUH	39	42.9	156	42.9
CHK	48	52.7	192	52.7
LNPMC	4	4.4	16	4.4
<i>Gender</i>				
Male	46	50.5	184	50.5
Female	45	49.5	180	49.5
<i>Education (years of schooling)</i>				
>12	10	11.0	52	14.3
6–12	28	30.8	108	29.7
≤5	53	58.2	204	56.0
<i>Profession<sup>a</sup></i>				
Housewife	39	42.9	153	42.0
Low earner	23	25.3	125	34.3
Medium earner	21	23.1	59	16.2
High earner	8	8.8	27	7.4
<i>Ethnicity</i>				
Punjabi	4	4.4	55	15.1
Sindhi	21	23.1	78	21.4
Mohajir	41	45.1	129	35.4
Baluchi	5	5.5	24	6.6
Pathan	4	4.4	27	7.4
Gujrati	5	5.5	28	7.7
Afghani/Makrani	11	12.1	24	6.2
<i>Age (completed years)</i>				
Mean (SD)	54.4 (13.4)		54.2 (13.9)	
Range	22–90		20–90	

AKUH = Aga Khan University Hospital; CHK = Civil Hospital Karachi; LNPMC = Liaquat National Postgraduate Medical Center.

<sup>a</sup> Low earner (rupees/month) ≤6000; medium earner (rupees/month) = 7000–20,000 and high earner (rupees/month) >20,000.

**Table 1b – Relative frequencies of various substances-use studied in relation to oesophageal squamous-cell carcinoma in a multicentre matched case–control study, Karachi, Pakistan.**

Risk factor <sup>a</sup>	Cases (n = 91)		Controls (n = 364)	
	n	%	n	%
Ever chewed areca nut alone	15	16.5	24	6.6
Ever chewed betel quid with tobacco	47	51.6	45	12.4
Ever practiced snuff dipping	12	13.2	27	7.4
Ever smoked cigarettes	31	34.1	89	24.5
Ever consumed alcohol	4	4.4	5	1.4

<sup>a</sup> Categorized yes versus no. None of the participants reported smoking hookah, cigar or pipe.

proportions of Mohajir (45.1% versus 35.4) and Afghan/Makrani (12.1% versus 6.2%) were represented, whereas, reverse was true for Punjabi ethnicity (4.4% versus 15.1%) (Table 1a). The proportions of various substance users were invariably higher among cases than the controls (Table 1b).

On univariable conditional logistic regression analyses, the variables significantly related with case/control status were ever chewed areca nut alone ( $p = 0.004$ ), ever chewed betel quid with tobacco ( $p < 0.001$ ), and ever smoked cigarettes ( $p < 0.038$ ). Other exposures marginally related with case/control status were ever practiced snuff dipping ( $p = 0.076$ ) and ever consumed alcohol ( $p = 0.070$ ). Also, Sindhi, Mohajir and Afghani/Makrani ethnicities were significantly ( $p < 0.05$ ) associated with oesophageal squamous cell carcinoma status on univariable analysis (Table 2).

Multivariable conditional logistic regression model showed that ever chewed areca nut alone ( $mOR_{adj} = 3.7$ ; 95% CI: 1.6–8.5;  $p = 0.002$ ), ever chewed betel quid with tobacco ( $mOR_{adj} = 12.8$ ; 95% CI: 6.3–26.2;  $p < 0.001$ ), ever practiced snuff dipping ( $mOR_{adj} = 4.3$ ; 95% CI: 1.6–11.7;  $p = 0.005$ ), ever smoked cigarettes ( $mOR_{adj} = 2.9$ ; 95% CI: 1.4–5.9;  $p = 0.005$ ) and Afghani/Makrani ethnicity ( $mOR_{adj} = 9.4$ ; 95% CI: 2.1–42.0;  $p = 0.003$ ) were significantly and independently associated with oesophageal squamous-cell carcinoma status (Table 3). The adjusted PAR (%) was the highest for ever chewed betel quid with tobacco (47.6), followed by ever smoked cigarettes (22.3), ever chewed areca nut alone (10.8) and ever practiced snuff dipping (10.1). The adjusted summary PAR (%) for all four substances together was 67.0 (Table 3).

None of the multiplicative interactions was significant (data not shown). However, the significant adjusted additive (biological) interaction effects revealed a multitude increase in the risk of oesophageal squamous-cell carcinoma, if the

respondents ever smoked cigarettes and ever chewed betel quid with tobacco ( $mOR_{adj} = 21.4$ ; 95% CI: 6.3–72.4;  $p < 0.001$ ) or if they ever smoked cigarettes and ever practiced snuff dipping ( $mOR_{adj} = 14.4$ ; 95% CI: 2.3–91.1;  $p = 0.005$ ) (Table 4). However, additive interaction effect of the dual practice of cigarette smoking and chewing areca nut alone on oesophageal squamous-cell carcinoma was statistically non-significant ( $p = 0.167$ ).

The adjusted PAR (%) was higher for the dual practice of cigarette smoking and chewing betel quid with tobacco (64.3) than the dual practice of cigarette smoking and snuff dipping (32.2) (Table 4). The synergy parameters' estimates were statistically non-significant, both for the dual practice of cigarette smoking and chewing betel quid with tobacco ( $RERI = 5.1$ ; 95% CI: –21.1–31.2;  $AP = 0.2$ ; –0.7–1.1;  $SI = 1.3$ ; 95% CI: 0.4–4.0), and for the dual practice of cigarette smoking and snuff dipping ( $RERI = 7.9$ ; 95% CI: –17.6–33.3;  $AP = 0.6$ , 95% CI: –0.2–1.4;  $SI = 2.6$ ; 95% CI: 0.3–21.1).

## 4. Discussion

### 4.1. Independent effects of substance use

In this multicentre matched case–control study, oesophageal squamous-cell carcinoma cases compared to controls were nearly four times more likely to be the chewers of areca nut alone. Comparable results were reported from India,<sup>21</sup> and Taiwan,<sup>22,23</sup> implicating areca nut chewing as an independent risk factor for oesophageal squamous-cell carcinoma. We did not enquire from the study participants about the habit of swallowing of areca nut juice while chewing. However, among the areca nut chewers congestion and erosion of the oesophageal mucosa has been recorded.<sup>24</sup> Therefore, it seems plausi-

**Table 2 – Univariable conditional logistic regression analysis of risk factors associated with squamous cell carcinoma of oesophagus in a multicentre matched case–control study, Karachi, Pakistan.**

Risk factor	Matched odds ratio	95% Confidence interval	p-Value
Ever chewed areca nut alone	2.8	1.4–5.8	0.004
Ever chewed betel quid with tobacco	7.6	4.4–13.3	<0.001
Ever practiced snuff dipping	2.0	0.9–4.1	0.076
Ever smoked cigarettes	1.8	1.0–3.3	0.038
Ever consumed alcohol	4.1	0.9–19.0	0.070

On univariable conditional logistic regression analysis, education, and income level were not significantly associated with case/control status. Whereas, on univariable analysis compared to Punjabi ethnicity, Sindhi ( $p = 0.001$ ), Mohajir ( $p = 0.007$ ) and Afghani/Makrani ( $p = 0.003$ ) were significantly more likely to be a case of oesophageal squamous cell carcinoma.

**Table 3 – Multivariable conditional logistic regression model of the risk factors associated with squamous cell carcinoma of oesophagus in a multicentre matched case–control study, Karachi, Pakistan.**

Risk factor	Cases with risk factor (%)	Controls with risk factor (%)	mOR <sub>adj</sub> <sup>a</sup>	95% CI <sup>a</sup>	p-Value	PAR (%) <sup>b</sup>
Ever chewed areca nut alone	16.5	6.6	3.7	1.6–8.5	0.002	10.8
Ever chewed betel quid with tobacco	51.6	12.4	12.8	6.3–26.2	<0.001	47.6
Ever practiced snuff dipping	13.2	7.4	4.3	1.6–11.7	0.005	10.1
Ever smoked cigarettes	34.1	24.5	2.9	1.4–5.9	0.005	22.3

<sup>a</sup> mOR<sub>adj</sub> = matched odds ratios; mORs<sub>adj</sub> are adjusted for ethnicity; CI = confidence interval.

<sup>b</sup> PAR (%) = population attributable risk percent; summary PAR (%) for four substances together was 67%.

**Table 4 – The combined effect of substance use on oesophageal squamous cell carcinoma in a multicentre case–control study, Karachi, Pakistan.**

Number of substances used		Cases n (%)	Controls n (%)	mOR <sub>adj</sub> <sup>*</sup>	95% CI <sup>**</sup>	p-Value	PAR (%) <sup>c</sup>
Ever smoked cigarettes	Ever chewed betel quid with tobacco						64.3
–	–	24 (26.4)	243 (66.8)	1.0	–	–	
+	–	20 (22.0)	76 (20.9)	3.7 <sup>a</sup>	1.6–8.5	0.002	
–	+	36 (39.6)	32 (8.8)	14.2 <sup>a</sup>	6.4–31.4	<0.001	
+	+	11 (12.2)	13 (3.6)	21.4 <sup>a</sup>	6.3–72.4	<0.001	
Ever smoked cigarettes	Ever practiced snuff dipping						32.2
–	–	52 (57.1)	254 (69.8)	1.0	–	–	
+	–	27 (29.7)	83 (22.8)	2.8 <sup>b</sup>	1.3–6.0	0.009	
–	+	8 (8.8)	21 (5.8)	4.1 <sup>b</sup>	1.3–12.9	0.018	
+	+	4 (4.4)	6 (1.6)	14.4 <sup>b</sup>	2.3–91.1	0.005	

\* Adjusted matched odds ratio.

\*\* Confidence interval.

<sup>a</sup> Adjusted for areca nut alone, snuff dipping and ethnicity.

<sup>b</sup> Adjusted for areca nut alone, betel quid with tobacco, and ethnicity.

<sup>c</sup> PAR (%) = population attributable risk percent.

ble that direct mucosal contact with swallowed areca nut juice might have contributed to the oesophageal squamous-cell carcinoma in our study. Previously several pathways have been suggested in the causation of oesophageal carcinoma by the constituents of areca nut. *In vitro* studies have shown that areca nuts' alkaloid – arecoline is a precursor for at least four N-nitrosamines and two of which are carcinogens.<sup>25</sup> Genotoxic and cytotoxic effects of areca nut extract and arecoline on various kinds of cells along with cell growth-inhibiting effects on gingival keratinocytes, oral fibroblasts and oral mucosa cells have been shown.<sup>26,27</sup> Moreover, it has been suggested that areca nut ingredients are critical in the pathogenesis of oral cancer via their stimulatory effects on cyclooxygenase-2 and prostaglandins,<sup>28</sup> which act as the inflammatory mediators for tumour initiation, promotion and metastasis.<sup>29</sup> Additionally, the constituents of areca nut may inhibit expression of the p53 tumour suppressor, impair DNA repair and activate matrix metalloproteinases-2, -8, and -9, which may accelerate tumour migration.<sup>30,31</sup>

We found a significant independent association between chewing betel quid with tobacco and oesophageal squamous-cell carcinoma. These results are consistent with those of previous studies, wherein chewing betel quid with tobacco was identified as a significant risk factor for oesophageal can-

cer.<sup>21,23</sup> Reportedly, the traditional habit of chewing and spitting out the contents of betel quid is changing to swallowing the betel quid juice and it is more common among South Asian immigrants in western countries.<sup>21</sup> Therefore, the direct contact with betel quid juice exposes oesophageal mucosa to potential carcinogens contained in various ingredients of the betel quid. As noted earlier, betel quid is a mixture of slaked lime (calcium hydroxide), with or without tobacco, areca nut, condiments and with and without sweeteners wrapped in a betel leaf.<sup>17,32</sup> Smokeless tobacco contains four specific nitrosamines that have been implicated for their role in oral, oropharyngeal and oesophageal carcinomas.<sup>16,33,34</sup> As stated earlier, areca nut contains a variety of alkaloids, mainly arecoline and 3-(methylnitrosamino) propionitrile; while lime provides reactive oxygen radicals, each of which has a role in carcinogenesis.<sup>34</sup>

Snuff dippers were at 4.3-fold increased risk of being oesophageal squamous-cell carcinoma cases than non-snuff dippers. Though 40% excess risk among snuff dippers was recorded but statistically non-significant association of snuff dipping with oesophageal squamous-cell carcinoma has been previously reported.<sup>35,36</sup> These opposing results may be due to varying composition and methods of preparation of oral snuffs used by the participants in this and previous studies.

Preparation of snuff by fermentation rather than heat processing may increase the concentration of tobacco-specific carcinogens.<sup>35</sup> We did not have the relevant evidence to support this contention. Previously, snuff dipping has been shown to be independently associated with oral cancer in Pakistan,<sup>17</sup> and USA.<sup>37</sup> The carcinogenic hazard of snuff dipping is of special concern due to its wide spread consumption by a large segment of South and Southeast Asian populations and their immigrants to western countries and need further evaluations.

Cases were nearly three times as likely to be ever-cigarette smoker as controls in this study. Cigarette smoking has been shown as an independent risk factor for oesophageal squamous-cell carcinoma.<sup>35,38,39</sup> and was more strongly related to squamous-cell than adenocarcinoma of oesophagus in earlier studies.<sup>35,40,41</sup> Numerous molecular alterations including cell-cycle regulation, growth factors and their receptors and DNA repair systems associated with carcinogenic effect of cigarette smoke have been proposed.<sup>39</sup> However, so far none has been underpinned as a specific mechanistic pathway leading to oesophageal squamous-cell carcinoma.<sup>39</sup>

#### 4.2. Combined effects of substance use

Additive (biological) interaction effects demonstrated a considerable increased risk of oesophageal squamous-cell carcinoma for those who indulged in the dual practice of cigarette smoking and chewing betel quid with tobacco or cigarette smoking and snuff dipping. This multifold increase in the risk of oesophageal squamous-cell carcinoma owing to the combined use of these substances in this study is in agreement with those reported earlier in the case-control studies from India,<sup>21,42,43</sup> Taiwan,<sup>22,23</sup> and a cohort study in Taiwan.<sup>44</sup>

The parameters' estimates for complete synergy between cigarette smoking and chewing betel quid with tobacco and between cigarette smoking and snuff dipping were statistically non-significant in this study. Incomplete synergy between cigarette smoking and chewing betel quid with tobacco or cigarette smoking and snuff dipping suggests that the use of either of the two substances increases oesophageal squamous-cell carcinoma risk in the absence of the other, but the combined effect of both exceeds the sum of the individual effects. Such incomplete synergy between the effects of these substances has also been observed earlier.<sup>22,43</sup>

The limitations of this study include: first, the selection of controls in a facility based case-control study is usually an issue. Although, we selected age, and sex matched controls nearly of similar socio-economic status for each case from the same hospital, yet controls from the inpatient or outpatient departments of the hospitals tend to be more similar to cases in their habits than general population. Therefore, the likelihood of under-estimation of effects of substances use may not be ruled out, though the direction of the bias would have been towards the null. Second, exposure ascertainment was based on self-reported information and recall bias is an inherent limitation of any case-control study. However, the initiation of these substances' use or quitting there-

after are perhaps quite defining moments in the life of the individuals, therefore, over and under reporting of duration and/or amount of the substances consumed was less likely to have occurred so sternly both among cases and controls. Third, interviewers were not blinded about the case or control status of the participants. However, to minimise interviewer's bias, they were trained in uniform delivery of questions to both cases and controls. Finally, this study showed a significant risk of oesophageal squamous-cell carcinoma associated with chewing betel quid with tobacco, chewing areca nut alone, and snuff dipping but we could not enquire about the swallowing of the juice of these substances while chewing. Nevertheless, our findings are nearly consistent with results of the earlier studies regarding the magnitude and direction of the effect estimates for these substances use either singly or in combination.

This study demonstrated significant independent associations of chewing areca nut alone, chewing betel quid with tobacco, snuff dipping and cigarette smoking with oesophageal squamous-cell carcinoma. Also, dual practice of cigarette smoking and chewing betel quid with tobacco or cigarette smoking and snuff dipping was associated with a significant multifold increased risk of oesophageal squamous-cell carcinoma. This study provided the adjusted PAR (%) for each substance use along with the adjusted summary PAR (%) for their combined use.

To our knowledge this is the first case-control study of predictors of oesophageal squamous-cell carcinoma in a country of 180 million population with up to 16% prevalence of smokeless tobacco use. The identified modifiable risk factors were involved in up to 67% of oesophageal squamous-cell carcinoma cases. Thus, taken together these results entail important implications for public health practice. Specifically, efforts to curtail the addiction to these substances may result in a substantial reduction in the incidence of oesophageal squamous-cell carcinoma and related mortality in this and similar settings.

#### Conflict of interest statement

None declared.

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